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Discovery

Development of a functional ghee incorporating Vallarai (*Centella Asiatica* (L.) urban

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ABSTRACT

The use of medicinal plants has been a central module of health care for centuries. Plants are always an exceptionally good source of drugs; many of the presently accessible drugs were directly extracted from plants. The curative properties of herbs have long been known and documented in ancient manuscripts, such as Sanskrit Rig Veda, Garuda Purana and Agni Purana. The use of *Centella* in food and beverages has increased over the years basically due to its health benefits such as antioxidant, as anti-inflammatory, wound healing, memory enhancing property and many others. This paper details the practical approach in preparing herbal ghee by incorporating the traditional knowledge along with the modern technology in drug manufacture.

Keywords: Vallarai (*Centella asiatica*), ghee, Ayurveda, traditional knowledge

1. INTRODUCTION

Ghee is a dairy product which has its origin in the Sanskrit word '*Ghrita'* meaning 'bright'. Beginning from the vedic times (3000 B.C. to 2000 B.C.), *makkhan* was extensively used by the early inhabitants of India; both in dietary and religious practices. Freshly

prepared ghee has a characteristic rich, nutty flavour and aroma. Its physical structure consists of somewhat granular appearance (Sserunjogi *et al.*, 1998). Fats provide Vitamins A, D, E, K and Essential Fatty Acids (EFAs) especially ω-3 and ω-6, which are proven anti-inflammatory eicosanoids. In general, fats nourish the skin, cell membrane and hair. It also protects the internal organs, maintain a healthy body temperature, store energy and nourish the brain.

Ghee is used as a medium in preparing various traditional medicines. Ghee, referred to as *Ghrita* in Ayurvedic science, is described as the best among lipids due to its quality of inheriting and enhancing potency of the drug it is enriched with. This property of ghee is deployed to use ghee as medicine where the lipids or fat property along with fat soluble chemical constituents of a particular drug are to be extracted for treatment (Shailaja *et al.*, 2013, Chitrangana *et al.*, 2014). Several '*Ghritam*' or ayurvedic medicated ghee are used for external and internal use. Medicated ayurvedic ghee is externally applied as dressing, ointment and enema. Internal use includes usage in ayurvedic *panchakarma* (therapeutic procedures). In Ayurvedic system of medicine, ghee is considered to induce several beneficial effects to human health and is used extensively for therapeutic purposes, such as in the preparation of a number of formulations for treating skin allergy and respiratory diseases, and is considered capable of increasing mental powers and physical appearance, and curative of ulcers and eye-diseases.

Drug Review

Centella Asiatica (vallarai) a clonal, perennial herbaceous creeper found throughout India growing in moist places up to an altitude of 1800 meters. Commonly known as "Gotu kola", it was historically known as "Snow plant" for the reason of its cooling properties (Emboden, 1985). In 1990, the estimated annual requirement of *C. Asiatica* was around 12,700 tonnes of dry biomass valued at Rs 1.5 billion (Ahmad, 1993). The plant was earlier confused with *Bacopa monnieri* Wettst., as both plants have been sold in the market by the name "Brahmi" (Anonymous, 1992). However, the Department of Indian System of Medicine and Homeopathy (ISM&H) have named that *Bacopa Monneria* is Brahmi (nira-Brahmi) and *Centella Asiatica* is Mandukparni (Bramhamanduki).

Botanical description of *Centella Asiatica*

It is a perennial, slender, herbaceous, creeper plant flowering between August and September. The plant has a smell reminiscent of tobacco and mildly bitter taste. The leaves are kidney shaped, 2-5 cm in diameter, with long petioles, arising from the stem nodes in rosettes. The stems (stolons) are slender, prostrate and often reddish coloured. The flowers are pale violet and bears fruits enclosed within a thick, hard pericarp (Jain, 1968).

Food uses

The use of *Centella* in food and beverages has increased over the years basically due to its health benefits such as antioxidant, as anti-inflammatory, wound healing, memory enhancing property and many others. *Centella* is traditionally used in summer drink popularly known as "thandaayee" (Anonymous, 2011). It is generally eaten as salad and ulam by Malay and Javanese people (Huda-Faujan *et al.*, 2007). In Thailand, *C. asiatica* leaves are blended and used in the form of cordial drink (Ilham, 1998) and also in tea and juice (Punturee, 2004). In Sri Lanka, leaves of *C. Asiatica* are used as a traditional curry and in the porridge to combat malnutrition (Cox *et al.*, 1993). Extract of the *C. asiatica* is also used in herbal noodles (Zainol, 2004). In China, it is used in the form of cooling drink (Turton, 1993, Zekaria and dan Mohd, 1994, Tiek, 1997). Leaves are also used to prepare chutney, hasuvale, tambali and toddy (Prakasha and Krishnappa, 2006).

Therapeutic uses

Centella Asiatica is a constituent part of the ayurvedic diet for diabetics. The most popular medicinal preparation is infusion, decoction, paste or juice. Whole plant is used for treating Leprosy, Epilepsy and Polyuria. *Centella Asiatica* is known to exhibit neuroprotective activity enhancing learning and memory (InduBala, 2009, Nasir *et al.*, 2011). Polyherbal formulation *Vallarai* chooranam is used to treat diabetics, urinary tract infection, leucorrhea, veneral disesase and also used to improve blood purification (Winston and Maimes, 2007). Ayurvedic medicine has effectively used *Centella Asiatica* in the treatment of inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly (Duke, 2001). In Chinese medicine, *C. Asiatica* is used for treatment of vomiting, epistaxis, urinary calculi, scabies and jaundice. Whole plant extract is reported to have anticancerous activity (Yu *et al.*, 2006). Methanolic extract of aerial parts of *C. Asiatica* inhibit the growth of human uterine carcinoma, human gastric carcinoma, and murine melanoma cells *in vitro* (Yoshida *et al.*, 2005). It has beneficial effects in treating anxiety and eczema (Hamid *et al.*, 2002). Alcoholic extract of the whole plant showed strong cardioprotective activity in limiting ischemia-reperfusion induced myocardial infarction in rats (Pragada *et al.*, 2004). Ethanolic and petroleum ether extracts show significantly high antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* (Jagtap *et al.*, 2009). Alcoholic extract showed antiprotozoal

activity against *Entamoeba histolytica* (Dhar *et al.*, 1968). Methanol extracts showed preliminary immunomodulatory effect (Jayathirtha and Mishra, 2004). Alcoholic extract showed the tranquilizing, sedative and antienxiety effects in experimental animal (Ramaswamy *et al.*, 1970). *Centella Asiatica* has strongest DPPH radical scavenging activity and highest total antioxidant capacity based on gallic acid and ascorbic acid equivalent among the eleven edible indian green leafy vegetables (Dasgupta and De, 2007).
Plant extracts can also act as effective antimicrobial agents that can be used alone or in combination in medicines or as natural food preservatives to retain the quality of food and prevent its spoilage. The antioxidant activity of Ethanolic extract of *Centella Asiatica* Linn can be attributed to the presence of active constituents such as terpenes such as Madecassic acid, Asiatic acid and three asiaticosides namely asiaticoside, asiaticoside A and asiaticoside B and phenolic constituents predominantly flavanoids and polyphenols. Phenolic compounds are known to have redox properties which help them act as hydrogen donators, reducing agents and singlet oxygen quenchers. In addition they also exhibit potent metal chelation potential. Polyphenols on the other hand have oxidation-reduction properties that play an important role in neutralizing free radicals (Brinkhaus, 2000; Obayed, 2009). These properties of active constituents in plants could be responsible for their antioxidant activity.

Conventional Pharmacodynamic properties of *Centella Asiatica* – (Tiwari *et al.,* 2011)

Rasa: *tikta* (bitter), *kasaya*, *madhura* (Bhavaprakasa nighantu), (Kaiyadeva nighantu)

Anurasa: *kasaya*, *madhura* (Bhavaprakasa nighantu)

Guna: *laghu*, *sara*

Virya: *sita* (Kaiyadeva nighantu)

Vipaka: *madhura*

Prabhava: *medhya*

Collection and Authentication of Raw Drugs

1 kg of identified and authenticated *Centella Asiatica* was collected from the local market of Erode, Tamilnadu state, India in the month of March 2015. 1 kg of fresh cream was procured from Milky Mist, Erode, India.

Figure 1 Leaves of *vallarai*

Figure 2 Soaking of extract

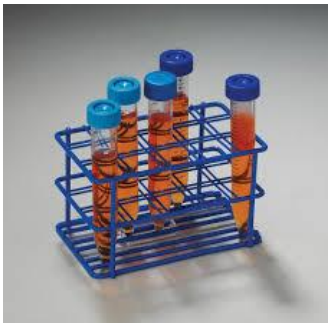

Figure 3 Extract in centrifuge tube

Figure 4 Centrifuge

Figure 5 Heating of butter

Figure 6 Frothy layer appearance

Figure 7 Curdling of cream

Figure 8 Ghee residue

Figure 9 Ghee in packed container

2. METHOD OF PREPARATION

The leaves were shade-dried for 2 days (fig. 1). It was then grinded and made into coarse powder using the mortar and pestle and was further pounded and made into fine powder using the mixer. Fine vallarai powder (75 g) was soaked in 3 ltr ethanol for overnight (fig. 2). Then the solution was centrifuged using ultra-centrifuge at speed of 1100 rpm for 5 mins (fig. 3, 4).The supernatant collected was evaporated at 60°C in a water bath. Then the extract was added to cream while melting stage came during heating process. During heating, stirring of the mixture was done. During the heating, a frothy layer appeared on the surface of the ghee (fig. 5) and the cream started curdling forming a solid consistency by continuous boiling (fig. 6, 7). A cohesive mud-like paste was formed at the bottom of the container, after which continuous stirring was done so as to avoid charring. The heating was continued till all the water evaporated from the ghee and the ghee started separating from the paste. The ghee formed a clear, transparent and devoid of any froth as the preparation was nearing the end point. The ideal ghee odour started to emanate confirmed that the entire water particle has evaporated. After obtaining this sign, the heating was stopped and the ghee was separated from the paste before cooling (fig. 8). The filtrate was collected in a clean sanitized vessel and was measured. 900 g of ghee was obtained. After cooling, ghee was measured and bottled in airtight containers (fig.9).

3. OBSERVATION

1.Yield of herb powder: From 1 kilogram of dried vallarai, 200 grams of fine powder was obtained.

2.Yield of ghee: 900 g ghee obtained from 1 ltr of cream.

3.Thick froth starts forming on the surface at around 25 mins of boiling.
4.Frothing considerably disappears by 5 mins, when the paste starts forming mud-like consistency. This is a sign of nearing end point as almost all water has evaporated. The crackling sound is very conspicuous when end point has not reached and becomes almost absent at endpoint.
5.*Ghrita* starts to separate after almost 20 mins after the paste attains mud like consistency.
6.200 grams of paste were obtained after the *ghrita* was separated from the mixture.

Sensory responses (Score) of optimized vallarai ghee and control ghee

Responses	Scores			
	Maximum	Predicted	Actual	Control
Flavour	50	44.1	40.3	46.5
Body and texture	30	22.7	21.6	27.5
Colour and appearance	10	8.4	8	8.6
Suspended solids	10	9.7	9	8.8
Overall acceptability	100	85.8	84.4	93.6

Study of anti-microbial activity

The plant materials are shade-dried, powdered with methanol using a mortar and pestle. The ground plant materials are centrifuged at 5000 rpm for 5 min and the supernatant is collected. Accurately weighed supernatant is dissolved in Dimethyl sulfoxide (DMSO) to the concentrations of 10 mg/ml, which is used of the anti-bacterial screening. On sterilised petriplates, LB (Luria-Bertani) agar is poured in equal quantities (about 25 ml) and dried. 50 µl of the bacterial culture is spread evenly with a sterile glass spreader on the LB agar dried on the petriplates and is dried. Wells of 6 mm diameter are bored on the prepared *E. Coli* culture using a sterile cork borer. It is then were filled with 100 µl of the extracts prepared and are then incubated at 37°C for 18-24 h. The diameter of zone of inhibition (mm) is calculated by subtracting the diameter of the well from the diameter of the circle in which the impact of the plant extract is seen. The methanolic extract shows significant antimicrobial activity against gram-negative bacteria *E. coli* as assessed by the diameter of zone of inhibition as 10 mm.

Radical scavenging activity

Chemicals and reagents: 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) and Quercetin was purchased from Sigma - Aldrich. Potassium Ferricyanide, Tricarboxyllic acid, Nitroblue Tetrazolium, Phenazine Methosulphate were purchased from Merck (Mumbai, India). Preparation of ethanolic extract: The leaves after procuring were shade-dried and coarsely powdered using mortar and pestle. Powder of *Centella Asiatica* Linn was subjected to extraction via the maceration technique with 1ltr ethanol each. The extract was subjected to a rotary flash to separate the alcohol from the plant extract. The obtained plant extract was further concentrated using water bath. The final extract was preserved in air desiccators. 0.1ml ethanolic extract of *Centella Asiatica* was mixed with 1.9ml of 200µM of DPPH and was allowed to stand for 20minutes for the reaction to occur. The absorbance was determined at 517 nm and from these values the corresponding percentage of inhibitions were calculated. The percentage inhibitions were plotted against respective concentrations used. Ascorbic acid was used as a positive control.

Total Antioxidant Activity (TAO)

Antioxidant activity of ethanolic extracts of *Centella Asiatica* compared with standard Quercetin. 0.2 ml of ethanolic extract of *Centella Asiatica* and 2 ml of reagent mixture (0.6 M of sulfuric acid, 28 mM sodium phosphate, 4 mM Ammonium molybdate in 1000 ml of water) was added and incubated at 95ºC for 90 minutes. Distilled water was used as blank. The procedure was repeated thrice and the absorbance was measured using UV spectrophotometer at 695 nm. Quercetin was used as positive control.

Test for Flavanoids

The ethanolic extract was dissolved in alcohol. Drops of 40% Sodium hydroxide solution were added and warmed. Dark yellow coloration indicated the presence of flavonoids.

Test for Phenols

To the ethanolic extract few drops of alcohol and ferric chloride solution were added. Bluish green coloration indicated the presence of phenols.

4. CONCLUSION

Herbal supplementation to enhance human physical performance has had little scientific study, but it represents a large and valid field for future study. Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds. There is recent increasing interest in alternative/herbal medicine for the prevention and treatment of various illnesses. As Ayurveda is foremost among the traditional health practices in the world, traditional inspired practical approach should be made in preparing prime quality preparations. The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders. Food fortification of commonly consumed foods may be a reliable and effective way to attain health benefits by increasing the nutrient intake of a population without relying on individual supplementation practices . The younger generation is conscious about their fitness and well being throughout and even elderly people are interested in delaying their aging process, all want to look young and vibrant forever. The solution lies in functional foods that provide stimulants rejuvenating vital body components through foods. People have now accepted the philosophy of "Prevention is better than cure" well in their day-to-day life.

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